Drug Discovery Strategies to Reduce the Selection of Antibacterial Resistance

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The Enemy*: Staphylococcus aureus*

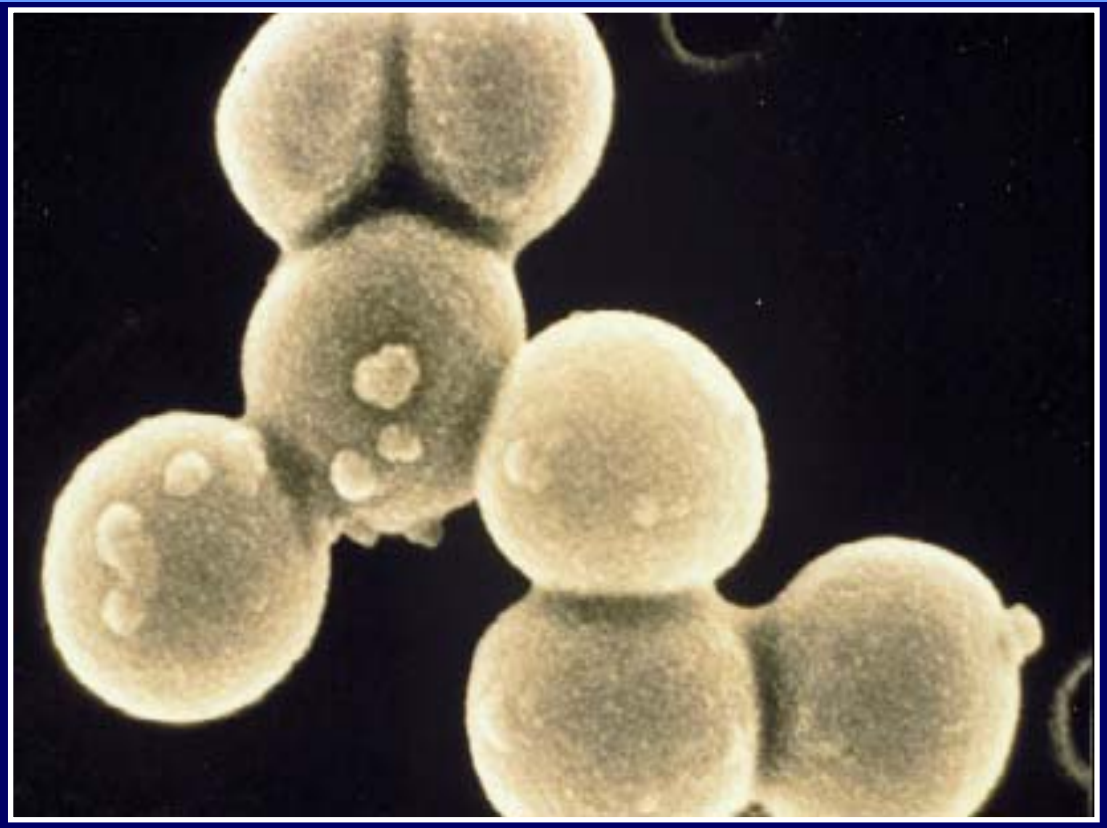


Photo Courtesy of Professor David Greenwood

Strengths	Weakness
Reproduce in 15’; Large numbers	Limited genome [predictable]
Mutation frequencies	Distinct from host, can be targeted

Overview of strategies to reduce resistance

- Resistance
  - definitions
  - importance
  - mechanisms
- Successful compound classes and targets characteristics
- Anti-bacterial strategies to overcome/reduce resistance
  - improvements in existing classes
  - dual target molecules
  - dual therapy
- Successful Drug Discovery Approaches

Definitions of antibiotic resistance

A mix of genetic, *in vitro,* and clinical parameters:

1. "… MICs fall in range where specific resistance mechanisms are likely…
2. "…isolates are not inhibited by the obtainable (blood) concentrations of the agent…"
3. "… Clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies"

- Definitions are vague with considerable subjectivity

- Clinical reports: MIC value > "breakpoint" value

CLSI M23 document, 2005

S. aureus resistance criteria

Drug	MIC_{90} (µg/mL)	Resistant (or NS) (µg/mL)	Fold above MIC_{90} (µg/mL)
Daptomycin	0.5	2	4x
Linezolid	1	4	4x
Vancomycin	2	16 or 32*	8x or 16x

*CLSI and FDA criteria, respectively

vancomycin resistance criteria out of alignment factor in rarity of vancomycin resistance reports

Antimicrobial Resistance in Nosocomial Pathogens, 2003

Drug/Pathogen	Resistance (%)
Methicillin/ *S. aureus*	57.1
Vancomycin/ enterococci	27.5
Quinolone/ *P. aeruginosa*	32.8
Methicillin/ CNS	89.1
3rd-gen. Ceph/*E. coli*	6.3
3rd-gen Ceph./*K. pneumoniae*	14.0
Imipenem/*P. aeruginosa*	22.3
3rd-gen. Ceph./*P. aeruginosa*	30.2
3rd-gen. Ceph./ *Enterobacter spp.*	32.2
Penicillin/*S. pneumoniae*	11.3

Source: CDC National Nosocomial Infections Surveillance System, August 2003 for all, except penicillin resistant *Streptococcus pneumoniae*, which is the Active Bacterial Core Surveillance of the Emerging Infections Network.

Resistance: Selection for survival

Moths, Birds, & Smokestacks – Manchester, UK

- Antibiotics don’t “cause” resistance [They select for it]

Resistance Mechanisms

Mechanism	Examples	Site of action
Inactivation	B-lactamases	Extracellular
Alteration of binding site	PBP2a, D-ala d-ala Gyrase mutations	Cell wall, cytoplasmic targets
Efflux	MDR pumps	Cytoplasm targets
Permeability	Mutations	Cytoplasm targets

Multiple mechanisms can function in a single bacterial strain
Function in both Gram positive and negative bacteria
Efflux and permeability protect internal targets

Discovery Strategies to minimize resistance

Good or easier

- Multiple targets and/or products of multiple genes
- Extracytoplasm targets (wall or membrane)
- Multistep mutations for resistance
- Whole cell screening
- Optimize PD

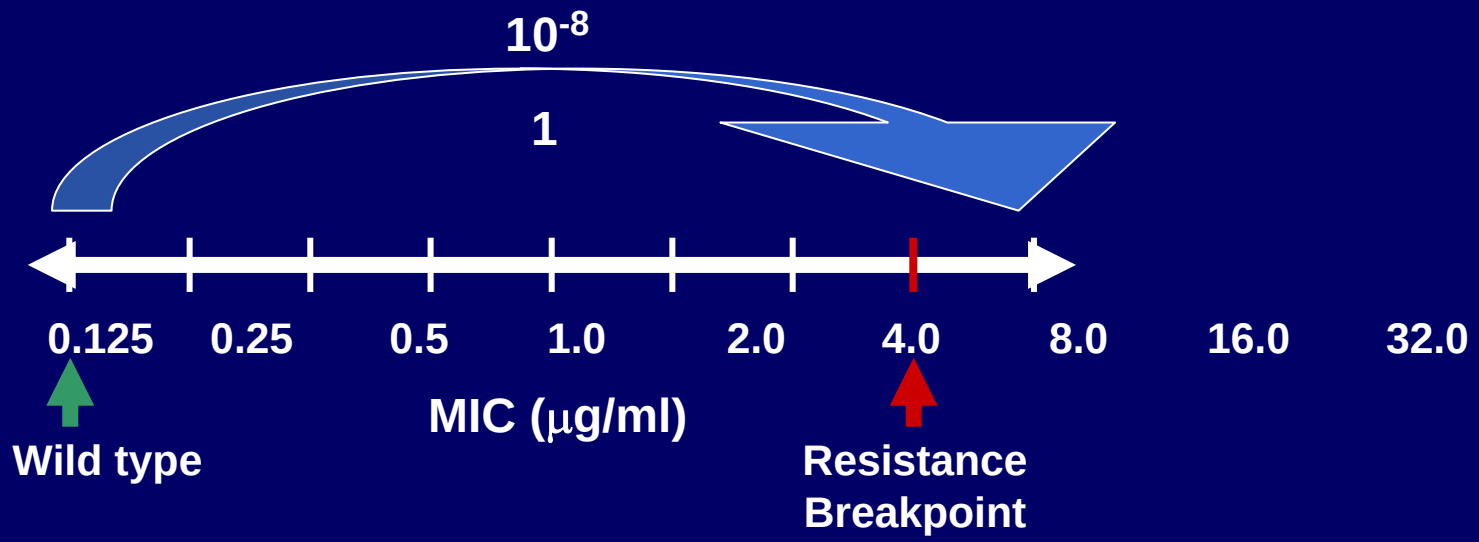
Poor or difficult

- Single target and single gene product
- Cytoplasm targets
- Single point mutations for resistance
- (only) In vitro target based screening

Resistance has developed to every class of anti-bacterial drug
Goal is to delay and minimize development of resistance

Single Targets/gene Antibiotics

- Worst if product of single gene
- No successful antibiotics targets product of single gene
- Several “single enzyme” antibiotics on market; all subject to high resistance rates (rifampin, novobiocin, trimethoprim)



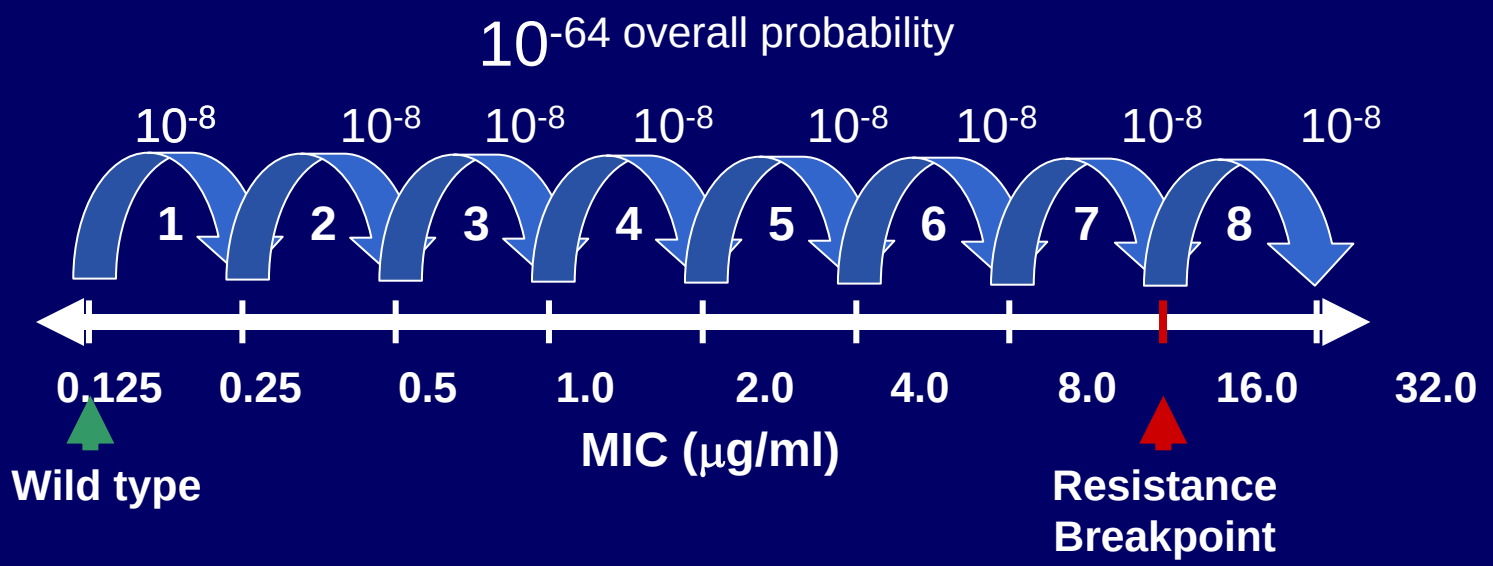
Single enzyme antibiotics

Drug	Mechanism	Resistance	Use
Fusidic Acid	Protein synthesis (EFG)	1x pt mutations Efflux	Combination
Novobiocin	DNA gyrase B	1x pt mutation	Combination
Rifampin	RNA polymerase	1x pt mutation RpoB	Combination
Sulfonamides	Folate synthesis (FolP)	Mutations in FolP, FolP production	Combination TMP
Trimethoprim	Folate synthesis (FolA)	Mutations in FolA, FolA production	Combination SMX

All are used only in combination to due to single target (resistance)

Multiple Targets

- products of multiple genes; multiple mutations required
- Several validated targets are all products of multiple genes
  - Penicillin binding proteins (PBPs)
  - Ribosomes (protein synthesis by rRNA)
  - Topoisomerases (replication)
  - Peptidoglycan structure (cell wall)
  - Bacterial membrane (polarity – replication?)



Validated targets

Target	Drug classes	Resistance inhibition
PBP -cell wall synthesis	B-lactams	Multiple PBP targeted
Ribosomes 16s or 23s – protein synthesis	Macrolides, tetracyclines, gentamicin, oxazolidones	Multiple gene operons
Topoisomerases – gyrase A+B, and topoisomerace IV	Fl-quinolones	Both gyrase (GyrA/B) and topoisomerase ParC/E targeted
Peptidoglycan – cell wall	Vancomycin	Multiple mutations required for cell wall
Membrane - depolarization	lipopeptides	Multiple mutations required

Perceptions of the Medical Community

"Caricature of the influenza epidemic of 1820"

Clin. Infect. Disease April 15, 2006

"French doctors celebrate the arrival of an influenza epidemic in 1820 … in anticipation of the money they will make…"

Next Generation of anti-bacterials

Improved versions of existing classes (additional targets)

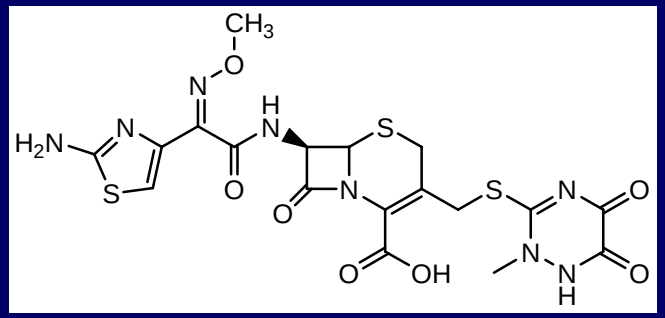
- Additional targets within class function
- MRSA cephalosporins, Fl-quinolones, carbapenams
  - ceftobiprole, ceftaroline

Dual target compounds (new target functionality added)

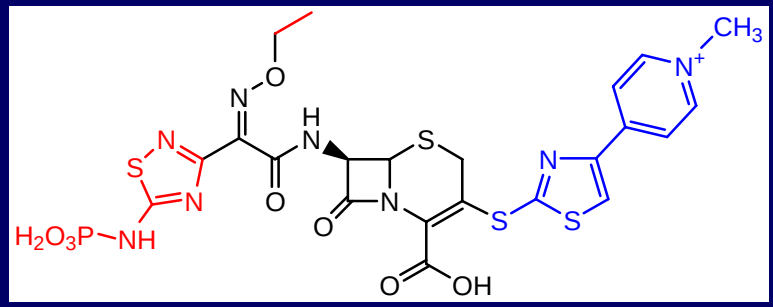
- single pharmacophore – dual targets
  - oritavancin, telavancin
- hybrid molecules
  - oxazolidinone/Fl-quinolone, rifampin/Fl-quinolones, glycopeptide/cephalosporin
- combination therapy
  - multiple classes

Improvement in existing classes:
MRSA cephalosporines

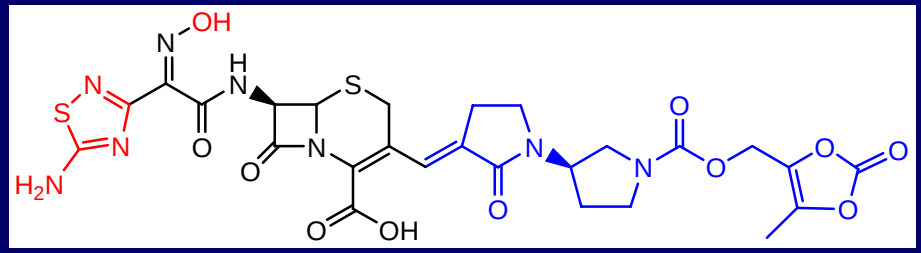
- Working within existing mechanisms
- ceftaroline, ceftobiprole target PBP2a + maintain binding to other critical PBP
- increased functionality through Medicinal Chemistry



Ceftriaxone



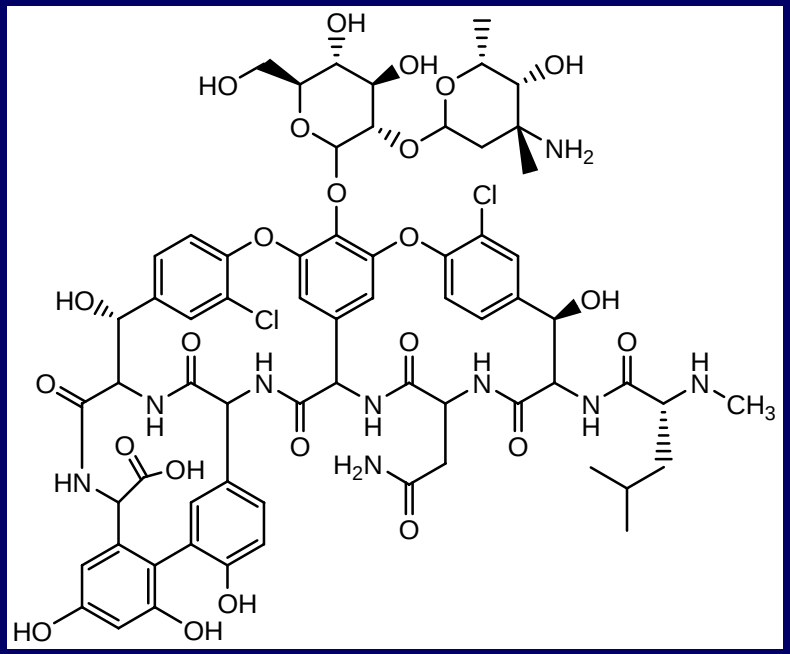
Ceftaroline



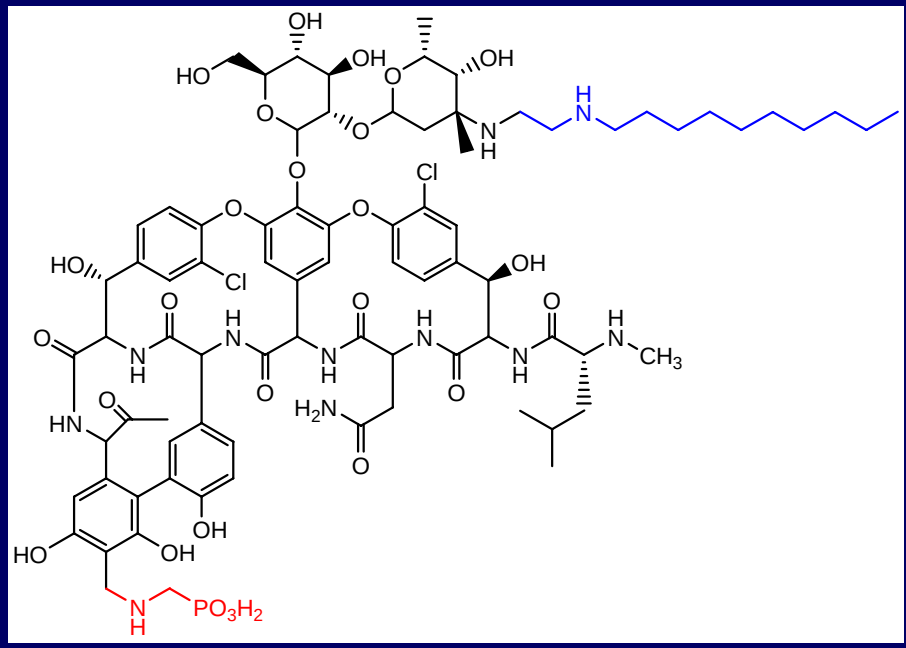
Ceftobiprole

Next Generation: Dual Targeting Compounds

- Telavancin, oritavancin, are vancomycin analogs
- Inhibition of peptidoglycan intermediates (D-ala D-ala) similar to vancomycon
- Additional functionality: membrane depolarization
- “lipoglycopeptides”



Vancomycin



Telavancin

Next Generation: Hybrid Molecules

- chemical linkage of two existing classes
- similar in concept to dual therapy
- Fl-quinolones popular choice due to relative degree of difficulty
- B-lactam/Fl-quinolones (Roche)
- oxazolidinone/Fl-quinolone (Biovertis)
- Rifamycin/Fl-quinolones (Cumbre)
- glycopeptides/cephalosporines (Theravance)

Next generation: Dual therapy

Dual therapies of different antibacterial targets already in use

- TPM/SMX
- dalfopristine/quinupristine
- many MTB therapies

Combination therapies to reduce bacterial resistance **Fish, *et al*. ***

- 173 studies covering over >14,000 patients
  - 8 antibiotic classes, 225 individual treatment regimens
- MIC value increased to resistance
  - 5.6% of all patients developed resistance
  - Higher in sequestered infections (osteomyelitis)
  - 0% in patients that received dual therapy

Pharmacotherapy 1995 15:279-291

Late stage antibacterial drugs

Drug	Class	Spectrum	Phase
Dalbavancin	Glycopeptide	G+	Approvable
Oritavancin	Glycopeptide	G+	Phase 3
Ceftobiprole	Cephalosporin	G+/G-	Phase 3
Telavancin	Glycopeptide	G+	Phase 3
Iclaprim	DHFR	G+	Phase 2
Ceftaroline	Cephalosporin	G+/G-	Phase 3
Cethromycin	Ketolide	G+	Phase 3
TD-1792	Glycopeptide/B-lactam	G+	Phase 2
PZ-601	Carbapenam	G+/G-	Phase 1

All are improved versions of existing drugs with dual targets or added targets

Role for Novel Targets

- Only two novel compounds/targets approved since 1970
- Only 14 drug classes total since 1935
- Novel targets are difficult to discover and develop
- Targets w/o natural inhibitors may be especially difficult

LPXc: critical pathway for LPS synthesis [Gram negative]

- intracellular, no known potent natural product inhibitors
- significant target-based effort produced in vitro inhibitors
- unable to date to develop pharmacology of compounds
  - solubility, PK, safety

History of Antibiotic Discovery and Approval

Year Introduced	Class of Drug
1935	Sulfonamides
1941	Penicillins
1944	Aminoglycosides
1945	Cephalosporins
1949	Chloramphenicol
1950	Tetracyclines
1952	Macrolides/Lincosamides/Streptogramins
1956	Glycopeptides
1957	Rifamycins
1959	Nitroimidiazoles
1962	Quinolones
1968	Trimethoprim
2000	Oxazolidinones
2003	Lipopeptides

Pre-1970: 12 classes
Post-1970: 2 classes

Source: Food and Drug Administration (modified)
Presented by John H. Powers, MD, at April 15-16, 2004 "Antimicrobial Drug Development Workshop," co-sponsored by FDA, IDSA, and the International Society of Anti-Infective Pharmacology.

Assessment of resistance potential

- Resistance incidence – single passage at 2x – 8x MIC value
  - survival ranges 10^5 (high resistance) to > 10^9 (low resistance)
- Serial passage
  - repeated passage at near MIC values over multiple days
  - no standard for evaluation; magnitude of MIC increase
- Heteroresistance
  - survival of subpopulations against a concentration gradient
  - no standard for evaluation
- Pharmacodynamic evaluation
  - resistance generation in simulation of clinical drug exposure
  - in vitro or animal model

Characteristics of Antibacterial Drug Discovery

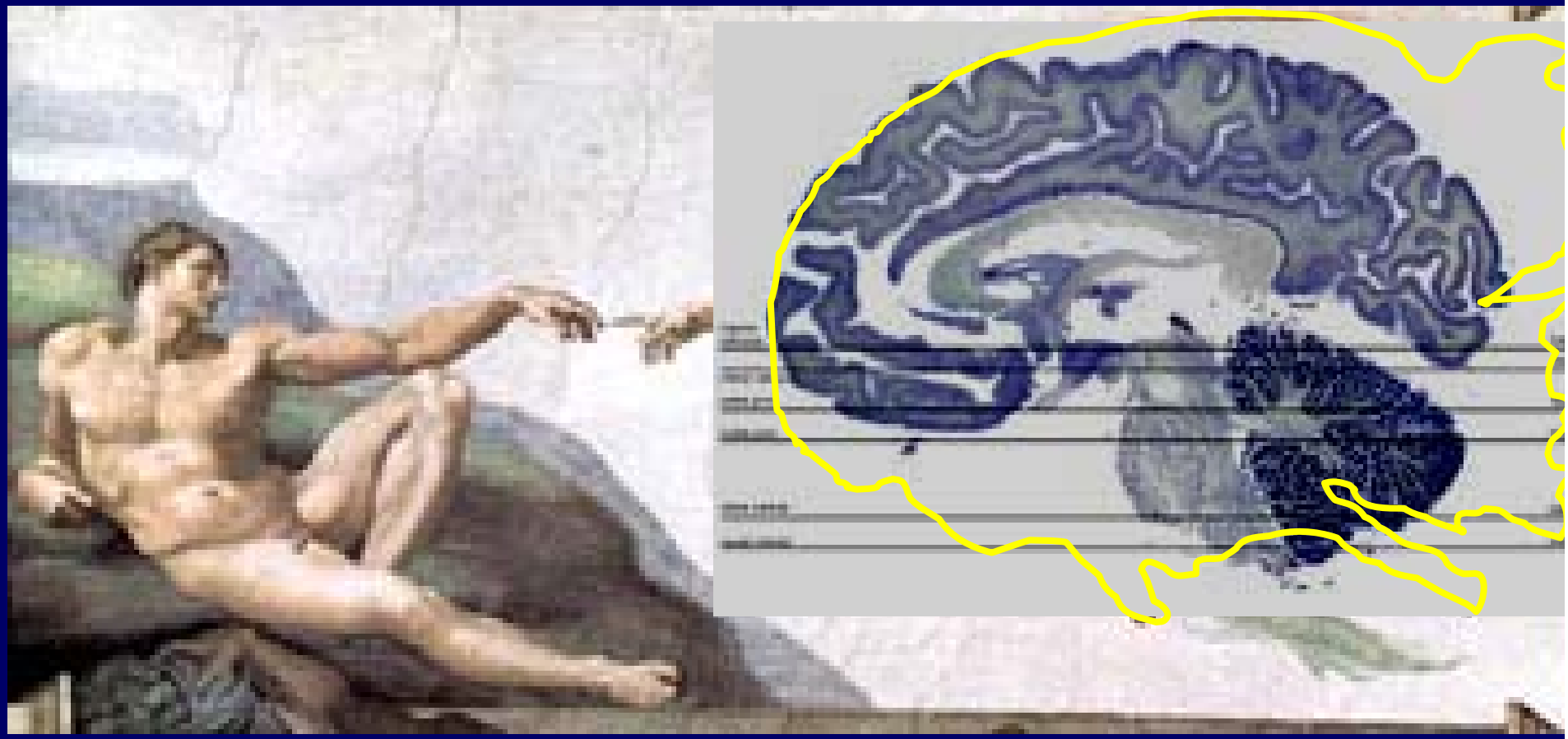
Function	Comments
Screening	Whole cell; avoid in vitro target based as sole SAR
Targets	Dual targets or at least product of multiple genes
Biology	Resistance assessed in multiple formats
Pharmacology	PD assessed for efficacy, toxicity, and reduction in resistance
Medicinal Chemistry	Critical function, ratio of 1:1 to 2:1 Chemist: biologist

Integration of functions is pivotal

Summary: Drug Discovery Strategies to Reduce the Selection of Antibacterial Resistance

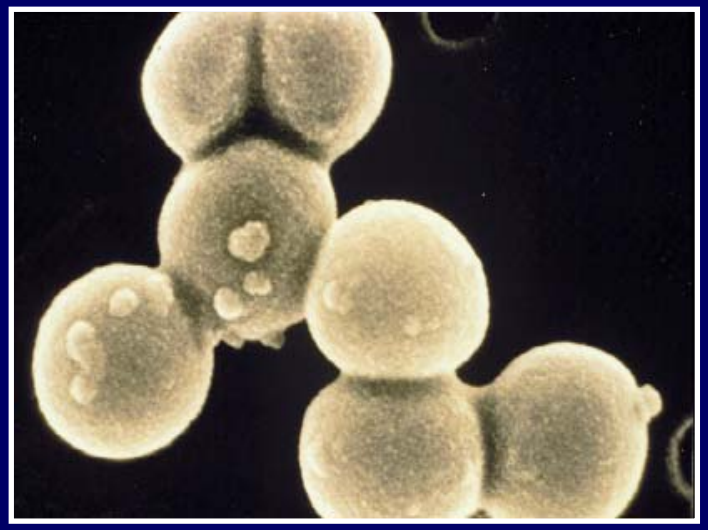
- Build Efforts around vetted targets/compound classes
  - multiple targets for pharmacophore
  - multiple genes for target
  - hybrid molecule with dual targets
- Consider resistance early in discovery program
  - assess in multiple formats
- Novel targets vetted for resistance etc
- Screen and test under pharmacologic conditions
- Use judgment on when to advance or kill program

Michelangelo: “The Creation of Adam”



“…and God created Man in his image, and Man was created In the Image of God”

The Final Analysis



1µm
1 billion years

Very large numbers
Random mutation
Ability to resist

2 meters
~50,000 years

Quality, not quantity
Directed purpose
Ability to create